Data Evaluation Report on the Chronic Toxicity of Chlormequat Chloride to Freshwater Invertebrates - Daphnia sp.

PMRA Submission Number {.....} EPA MRID No. 467152-15

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	D325185
	OECD Data Point	{.....}
	EPA MRID	467152-15
	EPA Guideline	850.1300

Test material: Chlormequat Chloride **Purity:** 63.3% ai
Common name Chlormequat chloride
Chemical name: IUPAC: 2-Chloroethyltrimethyl ammonium chloride
CAS name: Not reported
CAS No.: 999-81-5
Synonyms: Chlorocholine chloride

Primary Reviewer: Brian D. Kiernan **Date:** 06/20/2006
EPA/OPP/EFED/ERBIV

10/17/2006

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	031401	

Date Evaluation Completed: 06-04-2006

CITATION: Thun, S. 1993. 21 d Reproduction Test in Daphnia, Test article: "Chlormequat Chloride". Unpublished study performed by IBR Forschungs GmbH, Hannover, Germany. Laboratory Report No. 80-91-2309-05-93. BASF Registration Document No. 1993/1002328. Study submitted by BASF Corporation, Research Triangle Park, NC. Study initiated May 26, 1993 and submitted October 22, 1993.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the chronic toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 21-day-chronic toxicity of Chlormequat Chloride to *Daphnia magna* was studied under static renewal conditions. Daphnids were exposed to Chlormequat Chloride at nominal concentrations of 0 (negative control), 0.083, 0.30, 1.07, 3.86, 13.9, and 50.0 mg product/L. Adjusted for purity (63.3% ai), nominal concentrations were 0, 0.052, 0.19, 0.68, 2.44, 8.80, and 31.6 mg ai/L, respectively. Only three test levels (1.07, 3.86, and 13.9 mg product/L) were analyzed periodically for actual concentrations; the reviewer determined the time-weighted averages for these concentrations to be 0.337, 1.14, and 5.01 mg product/L (0.213, 0.722, and 3.17 mg ai/L). Results indicated that Chlormequat Chloride was not stable during the 2- to 3-day static periods, declining 53-81% of initial concentrations. In addition, a high level of analytical variability was observed in freshly-prepared solutions at the nominal 13.9 mg/L level, exceeding 20% of the mean value.

Following 21 days of exposure, cumulative mortality rates were 10, 0, 10, 5, 20, 30, and 100% in the 0 (control), 0.083, 0.30, 1.07, 3.86, 13.9, and 50.0 mg product/L test levels, respectively. No mortality occurred at levels equal to and lower than 3.86 mg product/L until day 19 of the study. The 21-day EC_{50} (with 95% C.I.) was determined to be 6.19 (4.33 to 8.86) mg product/L (3.92 (2.74 to 5.61) mg ai/L). The NOAEC for mortality was determined by the study author to be 1.14 mg product/L (0.722 mg ai/L), based on the time-weighted average concentration at this level.

No statistically-significant differences were observed on the overall mean number of offspring produced per day per adult; however, statistically-significant reductions in offspring production compared to the control were observed on day 16 at the 0.30 mg/L level (p= 0.0357), on day 9 at the 13.9 mg/L level (p=0.0458), and on days 16 and 19 at the 50.0 mg/L level (p=0.0005 and 0.0008, respectively). The NOAEC for reproductive success was 1.14 mg product/L (0.722 mg ai/L).

The time to first brood release was not assessed, and as observations were not performed daily, this endpoint could not be accurately assessed by the reviewer. A growth endpoint was not assessed.

This study is not adequate for use in risk assessments and is classified UNACCEPTABLE.

Results Synopsis

Test Organism Age (eg. 1st instar): First instar, <24 hours old
Test Type (Flow-through, Static, Static Renewal): Static renewal

21-day EC_{50} (survival): 6.19 mg product/L 95% C.I.: 4.33-8.86 mg product/L
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 1.14 mg product/L
LOAEC: 5.01 mg product/L

Adjusted for active ingredient:
21-day EC_{50} (survival): 3.92 mg ai/L 95% C.I.: 2.74-5.61 mg ai/L
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 0.722 mg ai/L
LOAEC: 3.17 mg ai/L

Endpoint(s) affected: Mortality and reproduction (only endpoints assessed)

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the OECD Guideline for Testing of Chemicals No. 202 (1984). Deviations from U.S. EPA OPPTS No. 850.1300 included:

1. The age, health (including mortality rates), and reproductive history of the laboratory cultures were not provided.

2. In this study, 40 daphnids were level were exposed per test level. The daphnids were divided into eight replicate vessels per level, each containing 5 organisms. This differs from OPPTS guidance, which recommends 10 or more replicates vessels (per level), each containing one daphnid.

3. The dilution water was not analyzed for particulate matter, TOC, ammonia, residual chlorine, metals, or pesticides.

4. Chlormequat Chloride was not stable under the static renewal conditions of the study, declining 53-81% of initial values during the 2- to 3-day static periods (reviewer-calculated). No discussion was provided by the study author indicating that this was a "best effort" by the laboratory to maintain constant concentrations.

5. Concentrations of Chlormequat Chloride in the freshly-prepared 13.9 mg/L solutions were highly variable, ranging from 7.92 to 14.24 mg product/L (mean concentration was 10.65 mg/L, with a 20% maximum allowable range of 8.52 – 12.78 mg/L). Concentrations were generally uniform at the two other tested levels (i.e., at 1.07 and 3.86 mg product/L).

6. Concentrations were not verified at each test level. This is especially important when the test material is known to volatilize or is unstable in test media.

7. The time to first brood release and a growth endpoint (total length or dry weight or both) were not determined.

The instability of the test substance under actual use conditions and the high level of variability in recoveries of Chlormequat Chloride in freshly-spiked samples affect the scientific soundness of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with GLP standards of the BGBI. I, No. 13, §19a (1990) and OECD (1982).

A. MATERIALS:

1. Test Material	Chlormequat Chloride
Description:	Clear, weakly yellow liquid
Lot No./Batch No. :	23/03/93
Purity:	63.3% [reviewer-calculated using 720 g ai/L product and physical density of 1.137 g/ml (provided in analytical report)]

Stability of compound under test conditions: Water samples were collected at the beginning and end of five of the 2 to 3-day periods of the renewal scheme for the nominal 1.07, 3.86, and 13.9 mg/L levels (actual days in relation to the study were not reported and could not be discerned). Chlormequat Chloride was not stable under the static renewal conditions of the study, declining 53-81% of initial values during the static periods (reviewer-calculated).

(OECD recommends water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Physicochemical properties of Chlormequat Chloride.

Parameter	Values	Comments
Water solubility at 20EC	>1 kg/kg	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

Storage conditions of Test chemicals: Protected from light, room temperature

2. Test Organism:

Species: *Daphnia magna*, first instar, <24 hours old
EPA and OECD recommend Daphnia magna

Age of the parental stock: Not reported
EPA recommends that young daphnids #24 hours old from a separate parental culture be used

Source: Laboratory cultures were maintained in synthetic water (Elendt Medium) under static renewal conditions (renewed three times/week). The daphnia were held in 1000 ml glass vessels, each containing approximately 30 animals. It was reported that at each renewal period, the newborn organisms were removed; otherwise, the age and reproductive history of the culture were not reported.
EPA requires all test organisms must be produced from laboratory reared culture that has been maintained for at least 21 days at test conditions in dilution water with renewal of the culture medium at least three times per week.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A 48-hour acute range-finding toxicity test was performed with first instar daphnids (6-24 hours old) at nominal concentration levels of 0 (negative control), 1.0, 1.8, 3.1, 5.6, 10.0, 17.7, 31.6, 56.2, and 100.0 mg product/L. Twenty daphnia were tested per level; the daphnia were divided into four replicates with five organisms/replicate. Each replicate vessel contained 10 ml of test solution.

The reference compound potassium chromate was concurrently tested at 0.4 and 1.4 mg/L. The daphnids were not fed during testing.

After 48 hours of exposure to Chlormequat Chloride, cumulative mortality was 5, 10, 55, and 95% in the 17.7, 31.6, 56.2, and 100 mg/L test levels, respectively. No mortality was observed at the lower levels or in the negative control group. The 48-hour EC_{50} (with 95% C.I.), calculated using Probit analysis, was 51.1 (41.8-71.5) mg/L.

After 48 hour of exposure to potassium chromate, mortality was 0% at the 0.4 mg/L level and 95% at the 1.4 mg/L level. Raw data were provided for the range-finding study.

At 0 and 48 hours, samples of test water from the nominal 0.032, 0.056, and 0.10 g/L Chlormequat Chloride levels were analyzed for ai using GC with flame-ionization detection (FID). After 48 hours, test substance concentrations declined 30, 10, and 36% from 0-hour recoveries in the 0.032, 0.056, and 0.10 mg/L solutions, respectively, indicating instability under the static renewal conditions of the study.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period:	Continuous	
Conditions: (same as test or not)	Same as test	
Feeding:	The daphnia were fed daily with unicellular green algae (*Scenedesmuss* spp.).	*EPA recommends that prior to testing, daphnids that are at least 10-12 days old (those that have had at least one brood) should be separated from the culture, put in separate container and maintained for at least 21 days to insure that good health conditions are present*
Health (any mortality observed):	Not reported	
Test condition: static renewal/flow-through:	Static renewal	
Type of dilution system- for flow through method.	N/A	
Renewal rate for static renewal	Every Monday, Wednesday, and Friday	*(EPA requires consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period)*
Aeration, if any	No aeration was described.	*EPA recommends test chambers should not be aerated*

Parameter	Details	Remarks *Criteria*
Duration of the test	21 days	*Recommended duration is 21 days.*
Test vessel Material: (glass/stainless steel) Size (for growth and reproduction/survival test): Fill volume:	Glass beakers 500 mL 250 mL	Five animals were maintained per replicate vessel. *1. Recommended Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Recommended Size: 250 ml with 200 ml fill volume; 100 ml with 80 ml fill volume* *OECD guideline recommends that parent animals be maintained individually; one per vessel, with 50 - 100 ml of medium in each vessel.*
Source of dilution water	Synthetic (reconstituted) water according to Elendt was prepared with purified water. The conductivity of the purified water was 0.049 µS/cm.	*Recommended source of dilution water includes unpolluted well or spring water that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness	2.5 mmol/L (sum of Ca^{2+}/Mg^{2+} - freshly prepared test water)	Dissolved oxygen levels exceeded 60% of air saturation (ca. 6.0 mg O_2/L).
pH	7.65-8.93 (measured in new and old test media)	***Recommended hardness:*** *160 to 180 mg/L as $CaCO_3$; OECD recommends > 140 mg/L as $CaCO_3$* ***Recommended pH:*** *7.6 to8.0 pH should not deviate by more than 1.0 unit for more than 48 hours. OECD recommends that pH range be 6 - 9 and does not vary more than 1.5 units in any one test.* ***Recommended dissolved oxygen:*** *renewal should not drop below 50% for more than 48 hours.* *Recommended flow-through: ∃ 60% throughout test.* ***Recommended temperature:*** *20EC ∀ 2EC.; should not deviate from 20EC by more than 5EC for more than 48 hours. OECD recommends a range of 18 - 22°C; temperature should not vary more than ∀ 2°C* *OECD guideline recommends that total organic carbon < 2 mg/L*
Dissolved oxygen	7.7-12.4 mg O_2/L (measured in new and old test media)	
Temperature	19.3-22.6°C (measured in new and old test media)	
Total Organic Carbon	Not determined	
Particulate matter	Not determined	
Metals	Not determined	
Pesticides	Not determined	
Chlorine		
Number of replicates	8 replicates per level	OPPTS guidance recommends that in static-renewal tests, 10 or more replicates of one daphnid each (per level). *Number of replicates should include a control(s) and at least 5 test concentrations; dilution factor should not be greater than 50%.* *OECD recommends that at least 5 test concentrations be used in a geometric series with a separation factor not exceeding 3.2.*

Parameter	Details	Remarks *Criteria*
Number of organisms: For growth and reproduction: For survival test:	5 per replicate 5 per replicate (same)	OPPTS guidance recommends that in static-renewal tests, 10 or more replicates of one daphnid each (per level). *Recommended number of organisms include 22 daphnids/test concentration;* *7 test chambers should contain 1 daphnid each, and 3 test chambers contain 5 daphnids each.* *OECD recommends holding a minimum of 10 daphnids individually for static tests.* *For flow-through tests, 40 animals should be divided into 4 groups of 10 animals at each test concentration.*
Treatment Concentrations: Nominal product: Nominal ai (63.3% of product): Measured ai:	0 (negative control), 0.083, 0.30, 1.07, 3.86, 13.9, and 50.0 mg product/L 0 (negative control), 0.052, 0.19, 0.68, 2.45, 8.80, and 31.6 mg ai/L 0.063 (nominal 0.68) mg ai/L 0.411 (nominal 2.45) mg ai/L 2.039 (nominal 8.80) mg ai/L Reviewer-calculated time-weighted averages from raw data; remaining levels were not measured.	Water samples were collected at the beginning and at the end of five of the 2 to 3-day periods of the renewal scheme for the nominal 1.07, 3.86, and 13.9 mg/L levels (actual days in relation to the study were not reported and could not be discerned). Chlormequat Chloride was not stable under the static renewal conditions of the study, declining 53-81% of initial values during the static periods (reviewer-calculated from raw data table). In addition, at the nominal 13.9 mg/L level, initial measured concentrations ranged from 7.92 to 14.24 mg product/L, exceeding 20% of the mean-measured limit (mean of 10.65 mg/L; limit range 8.52-12.78 mg/L).

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	N/A	*Solvent concentration should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone and ethanol. OECD recommends # 0.1 ml/L of solvent.*
Lighting	16 hours light/8 hours dark	Light intensity was approximately 1000 lux. *Recommended photoperiod is 16 hours light and 8 hours of dark.*
Recovery of chemical: Frequency of measurement: LOD: LOQ:	98.8 ± 11% A single analysis with four injections/level 0.2 mg ai/L 0.5 mg ai/L	Based on procedural recoveries. The LOQ was also referred to as the "determination limit".
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any Loading: Feeding:	The loading volume was 1 daphnid/50 ml solution. The daphnia were fed with algae (not further defined) at increasing COD levels. The initial rate was 4.4 mg/L COD per daphnia, and increasing during the study to 15.0 mg/L COD per daphnia.	It was reported that from day 17 through 21, the feeding volume was reduced (not further specified) because the daphnia did not consume the algae.

2. Observations:

Table 2: Observations

Parameters	Details	Remarks
		Criteria
Data endpoints measured (list)	- Survival of first-generation daphnids - Number of live/dead young produced per adult	A growth endpoint was not included. *Recommended endpoints measured:* *- Survival of first-generation daphnids,* *- Number of young produced per female,* *- Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test,* *- Observations of other effects or clinical signs.*
Observation intervals	Observations were made on days 0, 2, 5, 7, 9, 12, 14, 16, 19, and 21.	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Following 21 days of exposure, cumulative mortality rates were 10, 0, 10, 5, 20, 30, and 100% in the 0 (control), 0.083, 0.30, 1.07, 3.86, 13.9, and 50.0 mg product/L test levels, respectively. No mortality occurred at levels equal to and lower than 3.86 mg product/L until day 19 of the study. In addition, the study author reported that since mortality did not surpass the 20% threshold level, the NOAEC was 3.86 mg product/L. The 21-day EC_{50} (with 95% C.I.) was reported to be 11.5 (8.6-15.3) mg/L.

Table 3: Effect of Chlormequat Chloride on Growth and Survival of Daphnia sp.

Nominal Treatment mg product/L (and mg ai/L)	Mortality (dead or immobile)		Mean No. Offspring per day per adult	endpoint 3 (eg Time to first brood)	endpoint 4 (eg Length and weight)
	No. Dead	%			
Control (dilution water only)	36	10	7.27	N/A	N/A
0.083 (0.052)	40	0	7.63	N/A	N/A
0.30 (0.19)	36	10	6.96	N/A	N/A
1.07 (0.68)	38	5	7.10	N/A	N/A
3.86 (2.44)	32	20	7.04	N/A	N/A
13.9 (8.80)	28	30	7.07	N/A	N/A
50.0 (31.6)	0	100	---	N/A	N/A
NOAEC	3.86 mg product/L		3.86 mg product/L[(a)]	N/A	N/A
LOAEC	13.9 mg product/L		13.9 mg product/L	N/A	N/A

[(a)] NOAEC was based on statistically-significant reductions in offspring production compared to the control on day 9 at the 13.9 mg/L level (p=0.0458), and on days 16 and 19 at the 50.0 mg/L level (p=0.0005 and 0.0008, respectively). No statistically-significant differences were observed on the overall number of offspring per day per adult.

B. EFFECT ON REPRODUCTION:

No statistically-significant differences were observed on the overall mean number of offspring produced per day per adult; however, statistically-significant reductions in offspring production compared to the control were observed on day 16 at the 0.30 mg/L level (p= 0.0357), on day 9 at the 13.9 mg/L level (p=0.0458), and on days 16 and 19 at the 50.0 mg/L level (p=0.0005 and 0.0008, respectively). A statistically-significant reduction in offspring production was observed at the 0.30 mg product/L level compared to the control on day 16 (p=0.0357); however, as no statistically-significant differences were observed at the next two higher levels, this difference was not considered to be related to treatment. The NOAEC for reproductive success was 3.86 mg product/L.

The time to first brood release was not assessed, and as observations were not performed daily, this endpoint could not be accurately assessed by the reviewer. A growth endpoint was not assessed.

C. REPORTED STATISTICS:

The 21-day EC_{50} and associated 95% C.I. for the immobilization of adult daphnia were determined by the method of Spearman and Karber. The NOAEC for mortality was visually determined using a threshold comparison value of 20%, and was reported in terms of nominal product concentrations.

For reproduction, the number of dead offspring/number of adults and the number of alive offspring/number of adults were compared at each observation interval using a U-test (2-tailed, corrected for ties) according to Mann/Whitney. In addition, the overall mean number of offspring/day/adult was also analyzed, presumably using the same method. The NOAEC for reproductive endpoints were based on significance data, and were provided in terms of nominal product concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The 21-day EC_{50} was determined using the moving average method via Nuthatch statistical software, which provided a more sound 95% confidence interval than the Probit method. The NOAEC based on immobilization was visually confirmed; the large replicate size (i.e., 40) precluded the use of Fisher's Exact test to verify this value statistically. These calculations were performed using the reviewer calculated time-weighted averages for the three levels that were provided and nominal concentrations for all other levels. The reviewer calculated the number of offspring produced per adult per reproductive day; the reviewer's calculation of this endpoint differed from the study author's calculation because the reviewer considered the number of reproductive days as the number of days at which young appeared through test termination (ranging from 12 to 14 days for each replicate group). Data for this endpoint did not satisfy the assumptions of normality and homogeneity of variances, so the NOAEC was determined using the non-parametric Wilcoxon Rank Sum test with Bonferroni's adjustment via TOXSTAT statistical software.

21-day EC_{50} (survival): 6.19 mg product/L 95% C.I.: 4.33-8.86 mg product/L
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 1.14 mg product/L
LOAEC: 5.01 mg product/L

Adjusted for active ingredient:
21-day EC_{50} (survival): 3.92 mg ai/L 95% C.I.: 2.74-5.61 mg ai/L
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 0.722 mg ai/L
LOAEC: 3.17 mg ai/L

Endpoint(s) affected: Mortality and reproduction (only endpoints assessed)

E. STUDY DEFICIENCIES:

Chlormequat Chloride was not stable under the static renewal conditions of the study, declining 53-81% of initial values during the 2- to 3-day static periods (reviewer-calculated). No discussion was provided by the study author indicating that this was a "best effort" by the laboratory to maintain constant concentrations. Furthermore, highly variable recoveries of Chlormequat Chloride from freshly spiked test solutions were observed at the 13.9 mg/L level. Recoveries ranged from 7.92 to 14.24 mg product/L, with a mean of 10.65 mg/L. Recoveries among replicate chambers should not vary by more than 20% of the mean (resulting in a maximum allowable range of 8.52 – 12.78 mg/L). These deficiencies are considered major, and affect the scientific soundness of the study. Additional deficiencies from OPPTS 850.1300 guidance include failure to sample test solutions from all treatment levels and the control level, and failure to assess both the time for first brood release and a growth endpoint.

F. REVIEWER=S COMMENTS:

The reviewer's conclusion regarding the NOAEC value for reproductive rate was similar to the study author's. The reviewer's 21-day EC_{50} estimate was lower because the reviewer used the measured concentrations for the three

levels provided, while the study author used nominal concentrations to generate this value. The reviewer's results are reported in the Executive Summary and Conclusions sections.

Chlormequat Chloride was not stable under the static renewal conditions of the study, declining 53-81% of initial values during the static periods (reviewer-calculated). In addition, at the nominal 13.9 mg/L level, initial measured concentrations ranged from 7.92 to 14.24 mg product/L, which exceeds 20% of the mean (mean of 10.65 mg/L; limit range 8.52-12.78 mg/L).

The reviewer additionally calculated time-weighted averages for the three nominal concentrations that were analyzed (provided in the Appendix below). For the lowest analyzed concentration, the "old" solution concentrations were lower than the LOQ, so the average concentrations for this treatment were calculated using ½ the LOD (0.2 mg ai/L).

In the acute range-finding test, the LOD and LOQ for the GC method were 16 and 35 mg ai/L, respectively. However, the LOD was greater than test levels selected for use for the definitive test; therefore, analysis of the definitive study samples were subcontracted to a new laboratory. The method of analysis (GC/FID) was still used; however, the LOD and LOQ for definitive study samples decreased to 0.2 and 0.5 mg ai/L, respectively.

In-life dates for the definitive study were May 26 – June 16, 1993.

G. CONCLUSIONS:

This study is not scientifically sound and is thus unacceptable. Test concentrations were not sampled at each toxicant level and time to first brood release and a growth endpoint were not assessed. This study is classified as UNACCEPTABLE.

21-day EC_{50} (survival): 6.19 mg product/L 95% C.I.: 4.33-8.86 mg product/L
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 1.14 mg product/L
LOAEC: 5.01 mg product/L

Adjusted for active ingredient:
21-day EC_{50} (survival): 3.92 mg ai/L 95% C.I.: 2.74-5.61 mg ai/L
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 0.722 mg ai/L
LOAEC: 3.17 mg ai/L

Endpoint(s) affected: Mortality and reproduction (only endpoints assessed)

III. REFERENCES:

OECD. Guideline 202, Part II.

EEC. Directive 92/69.

DIN 38 412 Part 1.

Finney, D.J. 1971. Probit Analysis. Cambridge University, Third Edition.

Weber, E. Grundriβ der biologischen Statistik, 9. Aufl., Fischer, Jena S. 184-190.

Sachs, L. 1984. Angewandte Statistik, Springer Verlag. Auflage.

"Verlängerter Toxizitätstest bei Daphnia magna – Verfahrensvorschlag der ad hoc Arbeitsgruppe des Umweltbundesamtes Berlin".

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

	concentration	time	CT	
1.07 mg/L	0.96	1	0.96	
<LOQ	0.1	3	0.3	
	0.95	1	0.95	
<LOQ	0.1	3	0.3	
	0.94	1	0.94	
<LOQ	0.1	3	0.3	
	1.1	1	1.1	
<LOQ	0.1	3	0.3	
	1.29	1	1.29	
<LOQ	0.1	3	0.3	
		20	**6.74**	

TWA= 0.337

	concentration	time	CT	
3.86 mg/L	2.83	1	2.83	
	0.77	3	2.31	
	2.5	1	2.5	
	0.7	3	2.1	
	3.01	1	3.01	
	0.6	3	1.8	
	2.34	1	2.34	
	0.64	3	1.92	
	2.38	1	2.38	
	0.56	3	1.68	
		20	**22.87**	

TWA= 1.144

*for measured concentrations <LOQ, average was calculated using 1/2 LOD (0.2)

	concentration	time	CT	
13.9 mg/L	14.24	1	14.24	
	2.83	3	8.49	
	11.7	1	11.7	
	3.31	3	9.93	
	8.99	1	8.99	
	4.21	3	12.63	
	7.92	1	7.92	
	3.09	3	9.27	
	10.41	1	10.41	
	2.17	3	6.51	
		20	**100.09**	

TWA= 5.005

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SPAN          G              LC50          95 PERCENT CONFIDENCE LIMITS
 2            4.560171E-02   6.189542      4.328787  8.855552

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS    G              H             GOODNESS OF FIT PROBABILITY
 4             .3090064      3.391613       8.815289E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE     =      1.416804
95 PERCENT CONFIDENCE LIMITS  = .6292263      AND     2.204383
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LC50 = 5.05199
95 PERCENT CONFIDENCE LIMITS = 1.912705 AND 25.55337

LC10 = .6413265
95 PERCENT CONFIDENCE LIMITS = 7.263536E-02 AND 1.711872

reproductive rate
File: 5215r Transform: NO TRANSFORMATION

WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	control	11.276				
2	0.083	9.864	47.00	45.00	8	
3	0.3	10.500	53.50	45.00	8	
4	1.07	10.160	51.00	45.00	8	
5	3.86	12.950	66.00	45.00	8	
6	13.9	14.651	70.00	45.00	8	

Critical values use k = 5, are 1 tailed, and alpha = 0.05